

## MINIREVIEW

# MicroRNAs: New Players in Cardiac Injury and Protection

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## ABSTRACT

MicroRNAs (miRNAs) have emerged as a novel class of endogenous, small, noncoding RNAs that negatively regulate gene expression via degradation or translational inhibition of their target mRNAs. Over 700 miRNAs have been identified and sequenced in humans, and the number of miRNA genes is estimated at more than 1000. Individual miRNA is functionally important as a transcription factor because it has the ability to regulate the expression of multiple genes through binding to its target with imperfect or perfect complement. In the heart, miRNAs have been involved in several clinical scenarios, such

as ischemia/reperfusion (I/R) injury and heart failure suggesting that regulation of their function could be used as a novel cardioprotective strategy. In particular, miRNA-1, miRNA-21, miRNA-24, miRNA-29, miRNA-92a, miRNA-126, miRNA-133, miRNA-320, miRNA-199a, miRNA-208, and miRNA-195 have been shown to be regulated after I/R injury. Because tissue miRNAs can be released into circulating blood, they also offer exciting new opportunities for developing sensitive biomarkers, including miRNA-1, miRNA-126, miR-208, and miRNA-499, for acute myocardial infarction and other cardiac diseases.

## Introduction

miRNAs constitute a large class of phylogenetically conserved single-stranded RNA molecules of 19 to 25 nucleotides that are involved in post-transcriptional gene silencing. They arise from intergenic or intragenic (both exonic and intronic) genomic regions that are transcribed as long primary RNA transcripts by RNA polymerase II (Ambros, 2004; Bartel, 2004). Primary transcripts undergo two processing steps that produce the short “mature” molecule. The approximately 70-bp precursor miRNA product is processed by the enzymes Drosha and Dicer and their partner DGCR8/Pasha to generate a mature 22-bp product that binds mRNAs in a unique manner using Watson-Crick base pairing through a conserved 6- to 8-bp “seed” sequence as well as additional con-

tacts (Ambros, 2004). miRNAs regulate more than 30% of the protein-coding part of the human genome. More than 700 miRNAs have been identified and sequenced in humans (Friedman and Jones, 2009). The number of miRNA genes in the human genome is estimated to be more than 1000 (Bentwich et al., 2005), and some are shown to play a profound role in human development and disease. An individual miRNA is capable of regulating the expression of multiple genes because it can bind to its mRNA targets with either an imperfect or a perfect complement. This is an interesting characteristic because a single miRNA becomes functionally important as a transcription factor (Chen and Rajewsky, 2007). In general, their overall function is to modulate or fine-tune cellular phenotypes through repressing expression of proteins or to down-regulate protein expression when needed. Therefore, miRNAs could be the pivotal regulators in normal development and physiology, as well as disease states, including cancer and cardiovascular disease (Dalmay, 2008). miRNAs participate in many cellular processes, such as apoptosis (Chan et al., 2005; Cimmino et al., 2005; Xu et al., 2007), fat metabolism (Xu et al., 2003), cell differentiation

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**ABBREVIATIONS:** miRNA, microRNA; bp, base pair(s); I/R, ischemia/reperfusion; MI, myocardial infarction; HSP, heat shock protein; PTEN, phosphatase and tensin homolog deleted on chromosome ten; LV, left ventricle/left ventricular; Bim, Bcl-2 interacting mediator of cell death; CUGBP2, CUG triplet repeat-binding protein 2; COX-2, cyclooxygenase-2; NS-398, *N*-[2-(cyclohexyloxy)-4-nitrophenyl]-methanesulfonamide; DuP-697, 5-bromo-2-(4-fluorophenyl)-3-(4-(methylsulfonyl)phenyl)-thiophene; PC, preconditioning; HIF, hypoxia-inducible factor; PHD, prolyl hydroxylase; iNOS, inducible nitric-oxide synthase; AMI, acute myocardial ischemia; CK, creatine kinase.

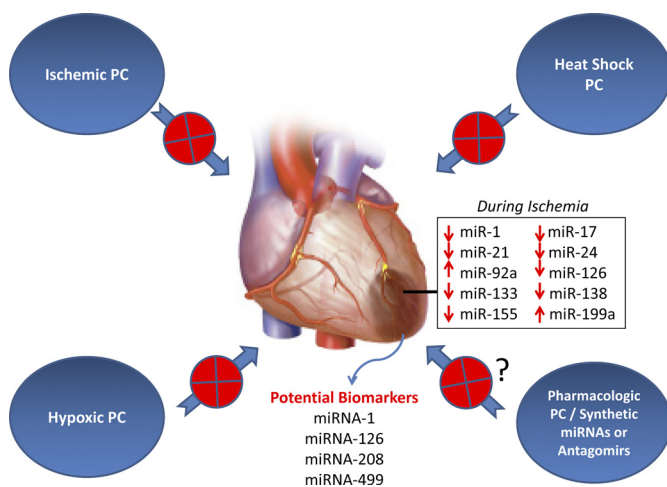
(Kwon et al., 2005; Silber et al., 2008; Tay et al., 2008), tumorigenesis (Chang et al., 2008), cardiogenesis (Chen et al., 2006; Rao et al., 2006; Zhao et al., 2007), and angiogenesis (Ji et al., 2007). This *Minireview* focuses primarily on the role of miRNAs in relation to myocardial ischemia/reperfusion (I/R) injury and cardioprotection (Fig. 1). We also discuss their potential use as biomarkers and/or therapeutic targets in myocardial infarction.

### microRNAs in Myocardial Infarction

Myocardial infarction (MI) continues to be among the most frequent causes of both debilitating illness and death. Tissue damage from MI occurs as a result of the initial ischemic event, determined primarily by its duration, and then subsequent injury resulting from reperfusion. During ischemia, ATP levels drop and intracellular pH is reduced as a result of anaerobic respiration and lactic acid accumulation. This in turn results in  $\text{Na}^+/\text{K}^+$ -ATPase dysfunction and consequent intracellular sodium build-up, leading to reversal of the  $\text{Na}^+/\text{Ca}^{2+}$  antiporter and increased cytosolic as well as mitochondrial calcium levels (Ostadal et al., 1999). Although  $\text{O}_2$  levels are restored at reperfusion, the respiratory chain complexes are in the reduced state, and a surge of reactive oxygen species is observed, particularly during the initial phase of reperfusion. The merger of all these adverse events orchestrates opening of the mitochondrial permeability transition pore, leading to cell death through necrosis and apoptosis (Kung et al., 2011). Mitochondria also constantly undergo fusion and fission, which are necessary for the maintenance of organelle fidelity (Youle and Karbowski, 2005; Suen et al., 2008; Tanaka and Youle, 2008). Abnormal mitochondrial fission is involved in the initiation of apoptosis, which requires Drp1, a GTPase that causes scission of the mitochondrial outer membrane, resulting in fission of mitochondrial tubules into fragments (Frank et al., 2001; Tan et al., 2008). Apoptotic signals can be mediated by calcineurin, a serine and threonine protein phosphatase that dephosphorylates pro- and antiapoptotic factors, leading to their activation or inactivation, respectively (Wang et al., 1999). In recent years, a number of studies have shown that up- or down-regulation of miRNAs contribute to cardiac injury and protection by

altering key signaling elements, thus making them potential therapeutic targets (Table 1). miRNA-1 is preferentially expressed in adult cardiomyocytes and skeletal muscle and is involved in cardiac development and heart disease. It is known to regulate a number of important functions, including apoptosis, by targeting the synthesis of HSP-60, HSP-70, and Bcl-2; arrhythmias by targeting *KCNJ2*, the gene encoding kir 2.1 (a subunit of the potassium ion channel) and *GJA1*, the gene encoding connexin 43 (a major component of the gap-junction) (Yang et al., 2007). Decreased expression of connexin-43 delays conduction, whereas decreased expression of kir 2.1 delays membrane repolarization, both of which may increase the risk of arrhythmias (Xu et al., 2007; Yang et al., 2007; Zhao et al., 2007; Tang et al., 2009). miRNA-21 is an interesting candidate for inhibition of apoptosis in the heart. It regulates the protein programmed cell death 4 and several putative cardioprotective mediators including activator protein-1, endothelial nitric-oxide synthase, HSP-70, and heat shock transcription factor-1 (Dong et al., 2009; Yin et al., 2009). Up-regulation of miR-21 also promotes cell survival through inhibition of PTEN (phosphatase and tensin homolog deleted on chromosome ten). PTEN degrades phosphatidylinositol-3,4,5-trisphosphate, which is produced by phosphoinositide 3-kinase and is essential for activation of the prosurvival Akt kinase pathway. miRNA-21 inhibition increased PTEN expression in cardiac fibroblasts, whereas miR-21 oligonucleotide suppressed it (Roy et al., 2009). It is noteworthy that miRNA-21 also controlled matrix metalloproteinase-2 expression via PTEN, suggesting the potential role of this miRNA in the treatment of heart failure and attenuation of inflammation. miRNA-24 expression is down-regulated in the ischemic border zone of the LV after ischemia and suppressed cardiomyocyte apoptosis, in part by direct repression of the Bcl-2-homology domain 3 (BH3)-only domain-containing protein Bim, which positively regulates apoptosis (Qian et al., 2011). Moreover, in vivo expression of miRNA-24 inhibited cardiomyocyte apoptosis, attenuated infarct size, and reduced cardiac dysfunction after MI. The antiapoptotic effect on cardiomyocytes in vivo was partially mediated by Bim.

The miRNA-29 family consists of three members (miRNA-29a, -29b, and -29c) that are transcribed from two bicistronic miRNA clusters. miRNA-29a and miRNA-29c may also have a role in modulation of I/R (Thum et al., 2007). Its potential targets are Mcl-1 (the antiapoptotic protein of Bcl-2 family), the regulatory subunit of phosphoinositide 3-kinase, p85a, and cell division cycle 42 (Park et al., 2009). Moreover, all three members of miRNA-29 up-regulate p53 levels and induce apoptosis in a p53-dependent manner. Antagomirs against miRNA-29a or miRNA-29c increased Mcl-2 expression and significantly reduced infarct size and apoptosis after I/R injury (Ye et al., 2010). Cardiomyocyte death after ischemia is followed by myocardial fibrosis, and several miRNAs have been linked to cardiac fibrosis: miRNA-29 expression is greater in cardiac fibroblasts than in cardiac-myocytes, and miRNA-29 is down-regulated in the border zone of infarcted hearts but still increases myocardial fibrosis by derepressing collagen and elastin translation (van Rooij et al., 2008). The biological function of miR-29 seems to involve fibrosis by targeting Elastin, fibrillin 1, collagen types I $\alpha$ 1 and I $\alpha$ 2, and collagen type III $\alpha$ 1. miR-92a increased 24 h after ischemia in mice (Bonauer et al., 2009). Antagomir-92a improved LV



**Fig. 1.** Schematic diagram showing dynamics of microRNAs during myocardial infarction and protective strategies including ischemic preconditioning, heat shock, hypoxia and pharmacologic preconditioning, with potential utilization of synthetic miRNAs and/or antagomirs.

TABLE 1  
Role of miRNA up- or down-regulation in cardiac injury and protection

miRNA	Up-Regulation	Down-Regulation	References
1	Increased apoptosis and arrhythmias	Decreased apoptosis and arrhythmias	Yang et al., 2007
133	Increased hypertrophy	Decreased hypertrophy	Xu et al., 2007; Bostjancic et al., 2010
21	Decreased apoptosis; cardioprotective	Increased apoptosis	Dong et al., 2009; Roy et al., 2009; Yin et al., 2009
24	Decreased apoptosis; cardioprotective	Increased apoptosis	Qian et al., 2011
320	Worsens I/R injury	Increased HSP20; cardioprotective	Fan et al., 2005; Ren et al., 2009
29	Increased apoptosis and fibrosis	Decreased apoptosis and fibrosis; cardioprotective	Thum et al., 2007; van Rooij et al., 2008; Park et al., 2009; Ye et al., 2010
92a	Decreased angiogenesis	Increased angiogenesis	Bonaue et al., 2009
126	Increased angiogenesis	Decreased angiogenesis	Harris et al., 2008; Wang et al., 2008
199a	Increased PHD2 and decreased HIF-1 $\alpha$	Decreased PHD2 and increased HIF-1 $\alpha$ ; cardioprotective	Rane et al., 2009
208	Dilated cardiomyopathy	Attenuates cardiomyopathy	Callis et al., 2009
499	Decreased apoptosis	Increased apoptosis	Wang et al., 2011

function, reduced infarct size, attenuated apoptosis, and increased angiogenesis in the border zone. Moreover, miRNA-92a targeted *Sirt1*, a class III histone deacetylase and longevity gene. Therefore, inhibiting miR-92a may potentially be important in improving remodeling and neovascularization after myocardial infarction. miRNA-126 is highly expressed in heart and lung tissue. It enhances the proangiogenic actions of vascular endothelial growth factor and fibroblast growth factor by promoting blood vessel formation via inhibition of the expression of Sprouty-related protein-1, an intracellular inhibitor of angiogenic signaling (Wang et al., 2008) and vascular cell adhesion molecule 1 (Harris et al., 2008). Knockdown of miRNA-126 during the embryonic development of zebrafish caused severe defects in vascular development, including collapsed blood vessels and cranial bleeding (Wang et al., 2008). It is noteworthy that miRNA-126 mutant mice with successful embryonic development demonstrated limited angiogenesis and decreased survival after myocardial infarction. miRNA-133 is expressed in adult cardiomyocytes and skeletal muscle. Patients who died of myocardial infarction had reduced levels of miRNA-133 in the infarcted areas of the heart (Bostjancic et al., 2010). miR-133 exerts antiapoptotic effects by targeting caspase 9 (Xu et al., 2007). Moreover, miRNA-133 was down-regulated with hypertrophy, and its up-regulation reduced hypertrophy as well as correction of conduction abnormalities. miRNA-320 has been shown to be down-regulated in mouse hearts after I/R injury (Ren et al., 2009). Overexpression of miRNA-320 enhanced cell death and apoptosis in cultured adult rat cardiomyocytes during simulated I/R, whereas its knockdown led to cytoprotection. Moreover, transgenic mice with cardiac-specific overexpression of miRNA-320 developed increased myocardial apoptosis and increased infarct sizes after I/R injury. The in vivo knockdown of miRNA-320 using an antagomir led to reduction of infarct size after I/R injury. Furthermore, miRNA-320 down-regulated HSP20, a protein that protects the heart against I/R injury (Fan et al., 2005), indicating HSP20 as a putative target of miRNA-320.

miRNA-499 is present abundantly in heart under physiological conditions (Kloosterman et al., 2006). miRNA-499 has been shown to be involved in inhibiting apoptosis and myocardial infarction induced by anoxia and ischemia (Wang et al., 2011) through mechanisms involving p53, calcineurin, and Drp1 in executing apoptosis program in the heart. Conversely, high doses of specific antagomir of miRNA-499 in-

duced apoptosis. These results suggest that modulation of miRNA-499 may be a novel therapeutic approach to treat apoptosis-related cardiac disease, including myocardial infarction.

Other interesting miRNAs are miRNA-208 and miRNA-195. Knockout of miRNA-208 reduced cardiomyocyte hypertrophy and fibrosis in a murine aortic banding model (Callis et al., 2009). In addition, transgenic mice with cardiac-specific overexpression of miRNA-195 developed a dose-dependent cardiac hypertrophy (van Rooij et al., 2006). In a recent study, Zhang et al. identified activation of miRNA-144/451 promoter by GATA-4, a critical transcription factor in the heart (Zhang et al., 2010). They observed that ectopic expression of miRNA-144 and miRNA-451 individually augmented cardiomyocyte survival, which was further improved by overexpression of miRNA-144/451, compared with control cells in response to simulated I/R. The knockdown of endogenous miRNA-144 and miRNA-451 revealed opposite effects. Using luciferase reporter assay and Western blot analysis, they validated that both miRNA-144 and miRNA-451 target CUG triplet repeat-binding protein 2 (CUGBP2), a ubiquitously expressed RNA-binding protein known to interact with COX-2 3'-untranslated region and inhibit its translation. Accordingly, protein levels of CUGBP2 were greatly reduced and COX-2 activity was markedly increased in miRNA-144-, miRNA-451-, and miRNA-144/451-overexpressing cardiomyocytes compared with green fluorescent protein-treated cells. Furthermore, inhibition of COX-2 activity by either *N*-[2-(cyclohexyloxy)-4-nitrophenyl]-methanesulfonamide (NS-398) or 5-bromo-2-(4-fluorophenyl)-3-(4-(methylsulfonyl)phenyl)-thiophene (DuP-697) partially offset the protective effects of the miRNA-144/451 cluster. These findings indicate that both partners of the miRNA-144/451 cluster confer partial protection against simulated I/R-induced cardiomyocyte death by targeting the CUGBP2-COX-2 pathway.

### miRNAs in Ischemic Preconditioning

Preconditioning (PC) is a phenomenon whereby the myocardium could be protected or "preconditioned" against prolonged ischemia by brief series of ischemia separated by bursts of reperfusion (Murry et al., 1986). The effect of PC appears in two phases: an early phase that lasts up to 2 h and a late phase, the effect of which persists as long as 96 h. The concept of PC has been of great interest to both the basic

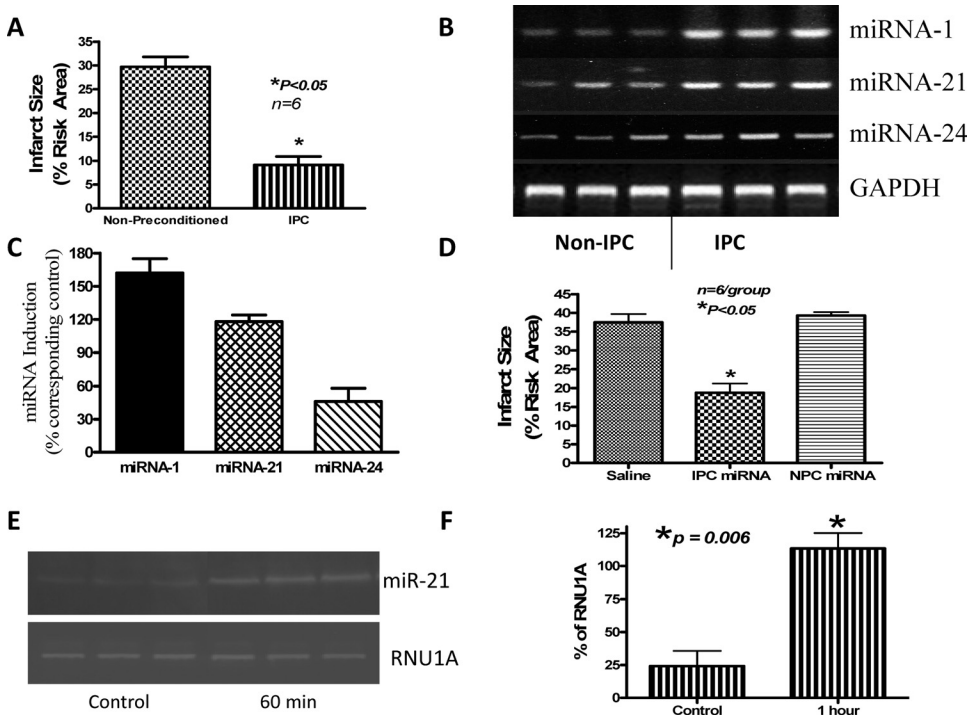


scientists and clinicians because it has helped discover numerous potential therapeutic options for cardioprotection, such as adenosine, opioid agonists, phosphodiesterase-5 inhibitors (Kukreja et al., 2005), nitrite (Lefer, 2009), carbon monoxide (Guo et al., 2004), remote conditioning, postconditioning (Xi et al., 2008) and, more recently, miRNAs (Yin et al., 2009). We isolated miRNAs after PC of the heart using short bursts of global ischemia and reperfusion, a stimulus that also caused significant reduction of infarct size in the Langendorff's isolated perfused heart model. The pool of miRNAs extracted from the nonpreconditioned and preconditioned hearts was injected into the risk zone of LV (area to be subjected to ischemia) in situ in another set of mice. After 48 h, the mice were subjected to I/R injury in vivo. The PC-derived miRNAs caused significantly lower infarct size ( $18.8 \pm 2.5\%$ ) compared with saline control ( $37.5 \pm 2.2\%$ ) or miRNAs prepared from nonpreconditioned hearts ( $39.3 \pm 2.3\%$ ). There was no difference in infarct size in the saline-injected control hearts versus nonpreconditioned miRNA-treated hearts (Fig. 2). It is noteworthy that there was significant up-regulation of eNOS protein ( $92 \pm 8.1\%$ ), heat shock transcription factor-1 ( $42.7 \pm 3.0\%$ ), and HSP-70 ( $102.3 \pm 8.9\%$ ) 48 h after treatment with miRNA derived from the PC hearts. However, there was no increase in iNOS protein, which is known to increase during late PC (Takano et al., 1998). Another study showed that miRNA-21 was down-regulated in the infarcted areas but up-regulated in the border areas of the heart after ischemia (Dong et al., 2009). Induction of PC clearly reversed these effects and inhibited the down-regulation of miRNA-21 in the infarcted areas. miR-1 has been linked to post-transcriptional repression of HSP-60 and HSP-70 in H9c2 cells (Xu et al., 2007), which is in contrast to the up-regulation of HSP-70 synthesis observed in our study. The difference in models (H9c2 cells versus murine intact hearts) may explain the discrepancy in the results between the two studies. miRNA-21 is a down-

stream effector of Akt and partly mediates its antiapoptotic effects through down-regulation of Fas ligand as well as caspase-8 (Sayed et al., 2010). This is important because PC also causes phosphorylation of Akt and protects the heart mainly through attenuation of apoptosis. Moreover, the inhibition of PTEN by miRNA-21 is consistent with the reported loss of PTEN activity after PC in the heart (Cai and Semenza, 2005).

### miRNAs in Cardioprotection with Heat Shock

Cells subjected to increases in temperature induce the expression of several proteins known as heat shock or stress proteins. This process enhances the cell's ability to overcome the effects of further stress. In fact, mild heat shock has been shown to improve myocardial survival after subsequent prolonged I/R injury (Currie et al., 1993). The synthesis of HSPs (Donnelly et al., 1992), antioxidant defenses (Karmazyn et al., 1990), enhanced mitochondrial respiration (Broderick, 2006), opening of mitochondrial  $K_{ATP}$  channel (Hoag et al., 1997), and subsequent resistance to opening of mitochondrial permeability transition pore (He and Lemasters, 2003) and inhibition of apoptosis have been considered as mechanisms of heat-shock protection against I/R injury. We recently demonstrated the role of endogenous miRNAs in heat shock-induced cardioprotection. We isolated the pool of miRNAs from liver and heart after heat-shock treatment in mice ( $42^\circ\text{C}$  for 15 min) and injected them into non-heat-shocked mice (Yin et al., 2008). Mice treated with the pool of miRNAs extracted from heat-shocked mice demonstrated improved ischemic tolerance. Infarct size was reduced significantly from  $40 \pm 2.7\%$  (percentage of total risk area, mean  $\pm$  S.E.M.) in the non-heat-shocked control mice to  $18.5 \pm 3.8\%$  in mice treated with miRNAs. Moreover, injection of chemically synthesized exogenous miRNA-21 also reduced infarct size by 64% ( $P < 0.05$  versus control). The miRNA-21-induced protection was totally abolished when mice were co-



**Fig. 2.** Induction of miRs by ischemic preconditioning (IPC) and Effect of miR on myocardial infarct size after ischemia/reperfusion. **A**, infarct size reduction after IPC ( $n = 6/\text{group}$ ). **B**, gel electrophoresis image of RT-PCR products of miRNAs. **C**, average normalized results showing increase in miR-1, miR-21, and miR-24 after IPC. The results are means  $\pm$  S.E.M. from three independent hearts. **D**, reduction of myocardial infarct size (percentage of risk area) after direct delivery of IPC-miRNA into the heart. \*,  $P < 0.05$  versus saline control and non-IPC miRNA-treated hearts. **E**, uptake of miR-21 after injection in LV wall. Gel electrophoresis image of the RT-PCR for miR-21. **F**, average normalized changes in miR-21. Endogenous U1A small nuclear RNA (RNU1A) was used as control. [Reprinted from Yin C, Salloum FN, and Kukreja RC (2009) A novel role of microRNA in late preconditioning: upregulation of endothelial nitric oxide synthase and heat shock protein 70. *Circ Res* 104: 572–575. Copyright © 2009 American Heart Association. Used with permission.].

treated with antagomir-21. miRNA treatment caused profound changes in several apoptosis-related genes as determined by gene microarray analysis. In particular, caspase family members 1, 2, 8, and 14 were suppressed in the hearts treated with miRNAs from heat-shocked mice. Except for *BNIP-3*, most of the proapoptotic genes, including *Bid* (Bcl-2-homology domain 3 interacting domain death agonist), *Bcl-10* (B-cell leukemia/lymphoma 10), *Cidea* (cell death-inducing DNA fragmentation factor,  $\alpha$  subunit-like effector A), *Ltbr* (lymphotoxin B receptor), *Trp53* (transformation-related protein 53), *Fas* (tumor necrosis factor receptor superfamily member) and *FasL* (Fas ligand, tumor necrosis factor superfamily, member 6), were repressed. On the other hand, the antiapoptotic genes *Bag-3* (Bcl-2-associated athanogene) and *Prdx2* (peroxiredoxin 2) were increased. These findings further support the notion that manipulating endogenous miRNA profiles could be a promising therapeutic approach to combat cardiovascular disease.

### miRNAs in Hypoxic Preconditioning

Similar to sublethal ischemia, hypoxia elicits PC-like response in which the myocardium develops an adaptive phenotype leading to resistance against subsequent lethal I/R injuries (Meerson et al., 1973; Bolli, 2000; Xi et al., 2002). This cardioprotection seems to be essentially mediated by hypoxia-inducible factor 1 $\alpha$  (HIF-1 $\alpha$ ) (Cai et al., 2003). HIF-1 is a heterodimeric  $\alpha/\beta$  transcription factor that mediates tissue responses to hypoxia (Wang and Semenza, 1993). HIF-1 is essential in the transcription of numerous genes that are implicated in oxygen homeostasis, especially in response to diminished oxygen tension. Prolyl hydroxylases (PHDs) hydroxylate HIF-1 during normoxia, which allows von Hippel-Lindau to bind and ubiquitinate HIF-1, marking it for proteasomal degradation. This process is inactivated during hypoxia, thus permitting rapid accumulation of HIF-1. Three PHD isoforms have been identified: PHD1, PHD2, and PHD3 (Bruick and McKnight, 2001; Ivan et al., 2002). PHD2 is ubiquitously expressed and exhibits the highest specific activity toward HIF-1 $\alpha$  (Hirsilä et al., 2003). Both PHD2 inhibition and hypoxia stabilize HIF-1 $\alpha$  protein and lead to increased levels of active HIF-1 $\alpha/\beta$  heterodimers in cell nuclei (Sutter et al., 2000). HIF-1 $\alpha$  stabilization by pharmacological inhibition of PHD2 or its silencing using a small interfering RNA strategy induced late PC like effect via an iNOS-dependent pathway (Ockaili et al., 2005; Natarajan et al., 2006). It has been shown that HIF-1 $\alpha$  is a predicted target of miR-199a and is rapidly induced by hypoxia (Rane et al., 2009). miR-199a interacted with the 3'-untranslated region of HIF-1 $\alpha$ , and inhibition of miRNA-199a increased HIF-1 $\alpha$  expression in cardiomyocytes. Moreover, hypoxic preconditioning or miR-199a silencing up-regulated iNOS and Bcl-2. Conversely, overexpression of miRNA-199a prevented up-regulation of iNOS during hypoxic preconditioning. Overexpression of miRNA-199a reduced Sirt1 levels, whereas silencing miRNA-199a by antisense oligonucleotide or down-regulating miRNA-199a by hypoxic preconditioning increased Sirt1 levels. miRNA-199a induced protection against I/R injury both by decreasing suppression of translation of HIF-1 $\alpha$  and by enhancing its stability via Sirt1-induced inhibition of PHD2. These studies suggest that miRNA-199a could be a valuable target for inducing PC.

### Antagomirs as Potential Drugs

Because miRNAs have been shown to be important in human disease, approaches to interrupt miRNA function may have therapeutic utility. Small interfering double-stranded RNAs engineered with certain "drug-like" properties, such as chemical modifications for stability and cholesterol conjugation for delivery, have been shown to achieve therapeutic silencing of an endogenous gene in vivo. To develop a pharmacological approach for silencing miRNAs in vivo, Krützfeldt et al. (2005) designed chemically modified, cholesterol-conjugated, single-stranded RNA analogs complementary to miRNAs and termed these oligonucleotides "antagomirs." Antagomirs targeting polycistronic miRNAs (miRNA genes that are located close together and are coordinately transcribed) were shown to retain their target specificity with no effect on the expression of cotranscribed miRNAs (i.e., antagomirs targeting miRNA-192 of the bicistronic cluster miR-192/194 resulted in the silencing of miRNA-192 with no effect on the expression levels of miRNA-194, and vice versa). Therefore, antagomirs have the ability to differentially silence specific miRNAs that derive from the same primary transcript. Moreover, they are valuable tools for identifying miRNA targets in vivo and for studying the biological role of miRNAs and miRNA-associated gene-regulatory networks in a physiological context. The therapeutic potential of antagomirs is also promising because mipomersen, an antisense oligonucleotide inhibitor of apolipoprotein B synthesis, demonstrated significant reductions in apolipoprotein B and low-density lipoprotein cholesterol in phase 1 clinical trials in healthy volunteers (Akdim et al., 2010). Mipomersen has also been tested in patients with familial hypercholesterolemia and showed exceptional results, with the highest efficacy seen so far in this patient population and with relatively low drop-out rates compared with other injectable drugs (Visser et al., 2010).

### MicroRNAs as Biomarkers

miRNAs may serve as potential diagnostic biomarkers for cardiovascular diseases because the serum or plasma can be obtained less invasively than tissue can. The endogenous circulating miRNAs have been found to be stable because of their packaging and secretion into the blood within the exosomes (Hunter et al., 2008). In the cancer field, miRNA-21 was the first miRNA suggested to be a diagnostic biomarker for diffuse large B cell lymphoma (Lawrie et al., 2008). Overexpressed miRNA-21 was associated with poor survival and poor therapeutic outcome in colon adenocarcinoma (Schetter et al., 2008).

In the cardiac field, an early and correct diagnosis of acute myocardial ischemia (AMI) is critical for rapid initiation of reperfusion therapy to reduce the irreversible damage in the heart and thus enhance survival of patients. Biomarkers such as creatine kinase-MB (CK-MB) isoenzymes, cardiac myoglobin, and troponins are now routinely used in clinical diagnosis (de Winter et al., 1995). Among these, cardiac troponins are currently considered the "gold standard" for AMI diagnosis (Jaffe et al., 2000). Unfortunately, many of these markers have reduced sensitivity, less specificity, or do not allow timely diagnosis. Therefore, multiple biomarker strategy may circumvent these limitations by adding accuracy and predictive power. A recent report suggests that serum

levels of cardiac-expressed miRNAs react to cardiac injury in a manner similar to that of cardiac enzymes. The plasma levels of miRNAs-208b and -499 have been shown to increase by more than 1000-fold after myocardial infarction, which mirrored the levels of troponin T (Corsten et al., 2010). Viral myocarditis was associated with 6- to 30-fold increases, whereas acute heart failure showed only a 2-fold increase of miRNA-499. Likewise, circulating levels of cell-free miRNA-1 were significantly increased in patients with AMI, which positively correlated with serum CK-MB levels (Cheng et al., 2010). Another clinical study showed that miRNA-1, miRNA-133a, and miRNA-208a in blood from patients with AMI were elevated compared with those from patients without AMI. Despite these encouraging results, the number of samples in the above-described studies is too small to provide definite proof of the diagnostic power of microRNA signatures and their value for clinical testing of AMI patients. Therefore, future prospective trials on large patient cohorts are needed to establish miRNAs as a novel biomarker class for AMI.

### Future Perspective

Similar to the first RNA revolution in the 1980s when the enzymatic activity of RNA was reported (Zaug and Cech, 1986), the discovery of miRNA represents the second RNA revolution (Kong and Han, 2005). Emerging evidence suggests that miRNAs are key regulators of cardiac diseases. Important new data suggest that miRNA-1, miRNA-133, miRNA-21, miRNA-24, miRNA-320, miRNA-29, miRNA-92a, miRNA-126, miRNA-199a, miRNA-208, and miRNA-195 seem promising and seem to play key roles in myocardial infarction, cardiac conduction, regulation of cardiac patterning, angiogenesis, cardiac hypertrophy, fibrosis, and cardiac protection. Analogous to protein kinases/phosphatases, the miRNAs could work independently or in concert to achieve certain outcomes. Intervention using stable miRNA mimetics and/or antagomirs is likely to emerge as a therapeutic tool for protection against AMI. The main advances in this exciting field will be in the following areas: 1) identifying novel microRNAs and their targets that regulate cardiac diseases, which would help in further understanding the mechanisms of cell death during I/R injury; 2) identifying the microRNAs that are changed particularly during the early phase after myocardial infarction and can be used as reliable markers for early diagnosis, improved prognosis, and treatment of ischemic heart diseases; and 3) defining the best conditions for the use of miRNAs and their inhibitors for reducing I/R injury/heart failure.

### Authorship Contributions

Wrote or contributed to the writing of the manuscript: Kukreja, Yin, and Salloum.

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